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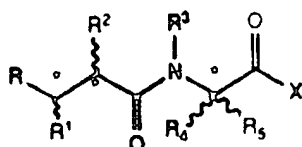
(71) Applicant : **CELLTECH LIMITED**  
**216 Bath Road**  
**Slough Berkshire SL1 4EN (GB)**

(72) Inventor : **Porter, John Robert**  
**6 Bader Gardens**  
**Slough Berkshire SL1 9EN (GB)**  
Inventor : **Millican, Thomas Andrew**  
**3 Harcourt Close, Dorney Reach**  
**Maidenhead, Berkshire, SL6 0DY (GB)**  
Inventor : **Morphy, John Richard**  
**27 Fomersby Crescent**  
**Maidenhead, Berkshire, SL6 1YY (GB)**  
Inventor : **Beeley, Nigel Robert Arnold**  
**16 Cheshire Road**  
**Thame, Oxfordshire, OX9 3LQ (GB)**

(74) Representative : **Hallybone, Huw George et al**  
**CARPMAELS AND RANSFORD 43 Bloomsbury**  
**Square**  
**London WC1A 2RA (GB)**

(54) **Peptidyl derivatives.**

(57) Compounds of formula (I) :



are described wherein R represents a -CONHOH, carboxyl (-CO<sub>2</sub>H) or esterified carboxyl group; R<sup>1</sup> represents an optionally substituted alkyl, alkenyl, aryl, aralkyl, heteroaralkyl or heteroarylthioalkyl group; R<sup>2</sup> represents an optionally substituted phenylethyl, phenylpropyl or phenylbutyl group; R<sup>3</sup> represents a hydrogen atom or an alkyl group; R<sup>4</sup> represents a hydrogen atom or an alkyl group; R<sup>5</sup> represents an optionally substituted alkyl or alkenyl group optionally interrupted by one or more -O- or -S- atoms or N(R<sup>7</sup>)- groups [where R<sup>7</sup> is a hydrogen atom or a C<sub>1-6</sub>alkyl group]; X represents an amino (-NH<sub>2</sub>), or substituted amino, hydroxyl or substituted hydroxyl group; and the salts, solvates and hydrates thereof.

The compounds are metalloproteinase inhibitors and in particular have a selective gelatinase action, and may be of use in the treatment of cancer to control the development of tumour metastasises.

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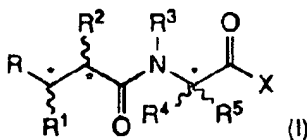
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In the formulae herein, the -line is used at a potential asymmetric centre to represent the possibility of R- and S- configurations, the  line and the  line to represent an unique configuration at an asymmetric centre.

In the compounds according to the invention, when the group R represents an esterified carboxyl group, it may be for example a group of formula  $-\text{CO}_2\text{R}^8$  where  $\text{R}^8$  is a straight or branched, optionally substituted  $\text{C}_{1-8}$  alkyl group such as a methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, s-butyl or t-butyl group; a  $\text{C}_{6-12}$ aryl $\text{C}_{1-6}$ alkyl group such as an optionally substituted benzyl, phenylethyl, phenylpropyl,  $\alpha$ -naphthylmethyl or  $\beta$ -naphthylmethyl group; a  $\text{C}_{6-12}$ aryl group such as an optionally substituted phenyl  $\alpha$ -naphthyl or  $\beta$ -naphthyl group; a  $\text{C}_{6-12}$ aryloxy $\text{C}_{1-6}$ alkyl group such as an optionally substituted phenyloxymethyl, phenoxyethyl,  $\alpha$ -naphthyloxymethyl or  $\beta$ -naphthyloxymethyl group; an optionally substituted  $\text{C}_{1-8}$ alkanoyloxy $\text{C}_{1-6}$ alkyl group, such as a pivaloyloxymethyl, propionyloxyethyl or propionyloxypropyl group; or a  $\text{C}_{6-12}$ aryloxy $\text{C}_{1-6}$ alkyl group such as an optionally substituted benzyloxyethyl or benzyloxypropyl group. Optional substituents present on the group  $\text{R}^8$  include for example one or more halogen atoms such as fluorine, chlorine, bromine or iodine atoms, or  $\text{C}_{1-4}$ alkyl, e.g. methyl or ethyl, or  $\text{C}_{1-4}$ alkoxy, e.g. methoxy or ethoxy, groups.

In general, when the group R represents as esterified carboxyl group, it may be a metabolically labile ester of a carboxylic acid.

When the group  $\text{R}^1$  in compound of formula (I) represents an optionally substituted alkyl or alkenyl group, it may be, for example, a straight or branched  $\text{C}_{1-6}$  alkyl or  $\text{C}_{2-6}$ alkenyl group such as methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, s-butyl, t-butyl, n-pentyl, i-pentyl, n-hexyl, ethenyl, 1-propenyl, 1-butenyl or 2-butenyl group optionally substituted by one or more  $\text{C}_{1-6}$ alkoxy, e.g. methoxy, ethoxy, propoxy,  $\text{C}_{1-6}$ alkylthio, e.g. methylthio, ethylthio, propylthio,  $\text{C}_{6-12}$ aryl $\text{C}_{1-6}$ alkoxy, e.g. phenyl $\text{C}_{1-6}$ alkoxy such as benzyloxy, aralkylthio, e.g. phenyl $\text{C}_{1-6}$ alkylthio such as benzylthio, amino( $-\text{NH}_2$ ), substituted amino, [such as  $-\text{NHR}^9$ , where  $\text{R}^9$  is  $\text{C}_{1-6}$ alkyl e.g. methyl or ethyl,  $\text{C}_{6-12}$ aryl $\text{C}_{1-6}$ alkyl, e.g. phenyl $\text{C}_{1-6}$ alkyl, such as benzyl,  $\text{C}_{6-12}$ aryl, e.g. phenyl,  $\text{C}_{3-8}$ cycloalkyl, e.g. cyclohexyl, or  $\text{C}_{3-8}$ cycloalkyl $\text{C}_{1-6}$ alkyl, e.g. cyclohexylmethyl group], carboxyl ( $-\text{CO}_2\text{H}$ ) or  $-\text{CO}_2\text{R}^8$  [where  $\text{R}^8$  is as defined above] groups.

Aryl groups represented by  $\text{R}^1$  in compounds of formula (I) include  $\text{C}_{6-12}$ aryl group such as phenyl or  $\alpha$ - or  $\beta$ -naphthyl groups.

Aralkyl groups represented by  $\text{R}^1$  include  $\text{C}^{6-12}$ aryl $\text{C}_{1-6}$ alkyl groups such as phenyl $\text{C}_{1-6}$ alkyl, or  $\alpha$ - or  $\beta$ -naphthyl $\text{C}_{1-6}$ alkyl, for example benzyl, phenylethyl, phenylpropyl, phenylbutyl, phenylpentyl,  $\alpha$ - or  $\beta$ -naphthylmethyl, naphthylethyl, naphthylpropyl, naphthylbutyl or naphthylpentyl groups.

When the group  $\text{R}^1$  in compounds of formula (I) is a heteroaralkyl group, it may be for example a  $\text{C}_{3-6}$ heteroaryl $\text{C}_{1-6}$ alkyl group, such as an optionally substituted pyrrolylmethyl, furanylmethyl, thienylmethyl, imidazolylmethyl, oxazolylmethyl, thiazolylmethyl, pyrazolylmethyl, pyrrolidinylmethyl, pyridinylmethyl, pyrimidinylmethyl, morpholinylmethyl, or piperazinylmethyl group.

Heteroarylthioalkyl groups represented by  $\text{R}^1$  include  $\text{C}_{3-6}$ heteroarylthio $\text{C}_{1-6}$ alkyl groups such as optionally substituted pyrrolylthiomethyl, furanylthiomethyl, oxazolylthiomethyl, thiazolylthiomethyl, pyrazolylthiomethyl, pyrrolidinylthiomethyl, pyridinylthiomethyl, pyrimidinylthiomethyl, morpholinylthiomethyl, or piperazinylthiomethyl groups.

The aryl, aralkyl, heteroaralkyl, or heterarylthioalkyl groups represented by  $\text{R}^1$ , and the groups represented by  $\text{R}^2$  in compounds of formula (I) may each optionally be substituted in the cyclic part of the group by one, two or more substituents [ $\text{R}^{10}$ ] selected from halogen atoms, e.g. fluorine, chlorine, bromine or iodine atoms, or  $\text{C}_{1-6}$ alkyl, e.g. methyl or ethyl,  $\text{C}_{1-6}$ alkoxy e.g. methoxy or ethoxy,  $\text{C}_{2-6}$ alkylenedioxy, e.g. ethylenedioxy, halo $\text{C}_{1-6}$ alkyl, e.g. tri-fluoromethyl,  $\text{C}_{1-6}$ alkylamino, e.g. methylamino or ethylamino,  $\text{C}_{1-6}$ dialkylamino, e.g. dimethylamino or diethylamino, amino ( $-\text{NH}_2$ ), nitro, cyano, hydroxyl ( $-\text{OH}$ ), carboxyl ( $-\text{CO}_2\text{H}$ ),  $-\text{CO}_2\text{R}^8$ , where  $\text{R}^8$  is as defined above,  $\text{C}_{1-6}$ alkylcarbonyl, e.g. acetyl, sulphonyl ( $-\text{SO}_2\text{H}$ ),  $\text{C}_{1-6}$ alkylsulphonyl, e.g. methylsulphonyl, aminosulphonyl ( $-\text{SO}_2\text{NH}_2$ ),  $\text{C}_{1-6}$ alkylaminosulphonyl, e.g. methylaminosulphonyl or ethylaminosulphonyl,  $\text{C}_{1-6}$ dialkylaminosulphonyl e.g. dimethylaminosulphonyl or diethylaminosulphonyl, carboxamido ( $-\text{CONH}_2$ ),  $\text{C}_{1-6}$ alkylaminocarbonyl, e.g. methylaminocarbonyl or ethylaminocarbonyl,  $\text{C}_{1-6}$ dialkylaminocarbonyl, e.g. dimethylaminocarbonyl or diethylaminocarbonyl, sulphonylamino ( $-\text{NHSO}_2\text{H}$ ),  $\text{C}_{1-6}$ alkylsulphonylamino, e.g. methylsulphonylamino or ethylsulphonylamino, or  $\text{C}_{1-6}$ dialkylsulphonylamino, e.g. dimethylsulphonylamino or diethylsulphonylamino groups. It will be appreciated that where two or more  $\text{R}^{10}$  substituents are present, these need not necessarily be the same atoms and/or groups. The  $\text{R}^{10}$  substituents may be present at any ring carbon atom away from that attached to the rest of the molecule of formula (I). Thus, for example, in phenyl groups any substituents may be present at the 2-, 3- or 4- 5- or 6- positions relative to the ring carbon atom attached to the remainder of the molecule.

When the groups R<sup>3</sup> and R<sup>4</sup> in compounds of formula (I) are alkyl groups, they may be for example C<sub>1-6</sub>alkyl groups such as methyl or ethyl groups.

The group R<sup>5</sup> in compounds of formula (I) may be an optionally substituted straight or branched C<sub>1-6</sub>alkyl, e.g. methyl, ethyl, n-propyl i-propyl, n-butyl, i-butyl, n-pentyl or n-hexyl or C<sub>2-6</sub>alkenyl e.g. ethenyl or 1-propenyl group optionally interrupted by one or more -O- or -S- atoms or -N(R<sup>7</sup>)- groups where R<sup>7</sup> is a hydrogen atom or is C<sub>1-6</sub>alkyl group such as a methyl group.

Optional substituents which may be present on alkyl or alkenyl groups R<sup>5</sup> include C<sub>6-12</sub>arylC<sub>1-6</sub>alkyl groups such as optionally substituted phenylC<sub>1-6</sub> e.g. benzyl groups, C<sub>6-12</sub>arylC<sub>1-6</sub>alkoxy groups such as optionally substituted phenylC<sub>1-6</sub>alkoxy e.g. benzyloxy groups, C<sub>6-12</sub>aryl e.g. optionally substituted phenyl groups, C<sub>3-8</sub> heteroaryl e.g. optionally substituted indole, imidazole or quinoline groups, C<sub>6-12</sub>arylC<sub>1-6</sub>alkoxyC<sub>6-12</sub>aryl, e.g. benzyloxyphenyl groups, -OH, -SH, C<sub>1-6</sub>alkylthio e.g. methylthio or ethylthio, carboxyl (-CO<sub>2</sub>H), amino (-NH<sub>2</sub>), carboxamido (-CONH<sub>2</sub>) or guanido -NHC(NH<sub>2</sub>)=NH, groups. The optional substituents present on these groups may be R<sup>10</sup> substituents as discussed above.

When X in the compounds of formula (I) represents a substituted amino group it may be for example a group of formula -NR<sup>11</sup>R<sup>12</sup>, where R<sup>11</sup> and R<sup>12</sup>, which may be the same or different, is each a hydrogen atom (with the proviso that when one of R<sup>11</sup> or R<sup>12</sup> is a hydrogen atom, the other is not) or an optionally substituted straight or branched alkyl group, optionally interrupted by one or more -O- or -S- atoms or -N(R<sup>7</sup>)- or aminocarbonyloxy [-NHC(O)O-] groups or R<sup>11</sup> and R<sup>12</sup>, together with the nitrogen atom to which they are attached, may form an optionally substituted C<sub>3-6</sub>cyclic amino group optionally possessing one or more other heteroatoms selected from -O- or -S-, or -N(R<sup>7</sup>)- groups.

when R<sup>11</sup> and/or R<sup>12</sup> is an alkyl group it may be for example a C<sub>1-6</sub>alkyl group such as a methyl, ethyl, n-propyl, i-propyl, n-butyl, i-butyl, s-butyl, or t-butyl group, optionally interrupted by one or more -O- or -S- atoms, or -N(R<sup>7</sup>)- or aminocarbonyloxy groups and may be for example a methoxymethyl, ethoxymethyl, ethoxymethyl, ethoxyethyl or ethylaminocarbonyloxymethyl group. The optional substituents which may be present on such groups include hydroxyl (-OH), carboxyl (-CO<sub>2</sub>H), esterified carboxyl (-CO<sub>2</sub>R<sup>9</sup>), carboxamido (-CONH<sub>2</sub>), substituted carboxamido, e.g. a group -CONR<sup>11</sup>R<sup>12</sup> where NR<sup>11</sup>R<sup>12</sup> is as defined herein, amino (-NH<sub>2</sub>), substituted amino, for example a group of formula -NR<sup>11</sup>R<sup>12</sup>, or aryl, e.g. C<sub>6-12</sub> aryl such as phenyl, optionally substituted by one, two or more R<sup>10</sup> substituents selected from those listed above in relation to the group R<sup>2</sup>.

Particular examples of cyclic amino groups represented by -NR<sup>11</sup>R<sup>12</sup> include morpholinyl, imidazolyl, piperazinyl, pyrrolol, oxazolyl, thiazolyl, pyrazolyl, pyrrolidinyl, pyridinyl and pyrimidinyl groups.

When the group X is a substituted hydroxyl group it may be for example a group -OR<sup>11</sup> where R<sup>11</sup> is as defined above, other than a hydrogen atom.

Salts of compounds of formula (1) include pharmaceutically acceptable salts, for example acid addition salts derived from inorganic or organic acids, such as hydrochlorides, hydrobromides, hydroiodides, p-toluene sulphonates, phosphates, sulphates, perchlorates, acetates, trifluoroacetates propionates, citrates, malonates, succinates, lactates, oxalates, tartarates and benzoates.

Salts may also be formed with bases. Such salts include salts derived from inorganic or organic bases for example alkali metal salts such as sodium or potassium salts, alkaline earth metal salts such as magnesium or calcium salts, and organic amine salts such as morpholine, piperidine, dimethylamino or diethylamine salts.

When the group R in compounds of the invention is an esterified carboxyl group, it may be a metabolically labile ester of formula -CO<sub>2</sub>R<sup>8</sup> where R<sup>8</sup> may be an ethyl, benzyl, phenylethyl, phenylpropyl, α- or β-naphthyl, 2,4-dimethylphenyl, 4-t-butylphenyl, 2,2,2-trifluoroethyl, 1-(benzyloxy)benzyl, 1-(benzyloxy)ethyl, 2-methyl-1-propionyloxypropyl, 2,4,6-trimethylbenzoyloxymethyl or pivaloyloxymethyl group.

In the compounds of formula (I) the group R<sup>1</sup> may in particular be a C<sub>1-6</sub>alkyl group such as a methyl group, an aralkyl group such as benzyl group, an arylthioalkyl group such as a phenylthiomethyl group or a heteroarylthioalkyl group such as thienylthiomethyl, piridinylthiomethyl or pyrimidinylthiomethyl group or is especially a hydrogen atom.

The groups R<sup>3</sup> and R<sup>4</sup> in compounds of formula (I) may each in particular be a methyl group, or, especially, a hydrogen atom.

The group R<sup>5</sup> in compounds of formula (I) may in particular be a C<sub>1-6</sub>alkyl group, e.g. an i-propyl or i-butyl group, or an optionally substituted benzyl, benzyloxybenzyl or indolymethyl group.

The group X in compounds of formula (I) may be in particular amino (-NH<sub>2</sub>) or -NR<sup>11</sup>R<sup>12</sup> group. Particular -NR<sup>11</sup>R<sup>12</sup> groups are -NHR<sup>12</sup> groups. Groups of this type include those where R<sup>12</sup> is a C<sub>1-6</sub>alkyl group, for example a methyl, ethyl, or n-propyl group, optionally interrupted by one or more -O- or -S- atoms or -N(R<sup>7</sup>) [e.g. -NH- or -N(CH<sub>3</sub>)-] or aminocarbonyloxy groups and optionally substituted by a hydroxyl, carboxyl, carboxyalkyl, e.g. carboxymethyl, carboxamido, amino, -NR<sup>11</sup>R<sup>12</sup>, [for example di-C<sub>1-6</sub>alkylamino such as

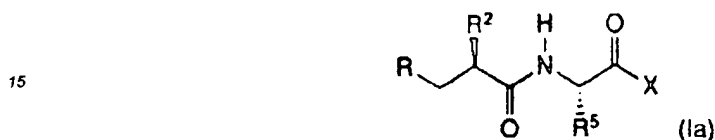
dimethylamino, C<sub>1-6</sub>alkylamino such as methylamino, or C<sub>3-6</sub> cyclic amino such as morpholinyl, pyrrolidinyl or pyridinyl] or phenyl optionally substituted by one, two or more R<sup>10</sup> substituents.

A particularly useful group of compounds according to the invention is that of formula (I) where R<sup>2</sup> is an optionally substituted phenylpropyl group.

5 A further particularly useful group of compounds of formula (I) are those wherein X is an amino or substituted amino group.

In general, in compound of formula (I) the groups R<sup>1</sup>, R<sup>3</sup> and R<sup>4</sup> is each preferably a hydrogen atom.

In a further preference, the group R in compounds according to the invention is a -CONHOH or a -CO<sub>2</sub>H group or a metabolically labile ester thereof. In a particular preference, however, R is a -CONHOH or a -CO<sub>2</sub>H group. An especially useful group of compounds according to the invention has the formula (Ia)



wherein R, R<sup>2</sup>, R<sup>5</sup> and X are as defined for formula (I); and the salts, solvates and hydrates thereof.

20 A particularly useful group of compounds of formula (Ia) are those wherein R represents a -CONHOH or -CO<sub>2</sub>H group; R<sup>2</sup> represents an optionally substituted phenylpropyl group; X is an amino (-NH<sub>2</sub>) or substituted amino group; and the salts, solvates and hydrates thereof.

In the compounds of formula (Ia) X may be a -NH<sub>2</sub> group or a group -NR<sup>11</sup>R<sup>12</sup> as defined for compounds of formula (I). The group R<sup>5</sup> may in particular be a C<sub>1-6</sub>alkyl group such as an i-propyl or i-butyl group, or an optionally substituted benzyl, benzyloxybenzyl or indolymethyl group.

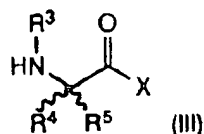
25 The compounds according to the invention may be prepared by the following processes. In the description and formulae below the groups R, R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>5</sup> and X are as defined above, except where otherwise indicated. It will be appreciated that functional groups, such as amino, hydroxyl or carboxyl groups, present in the various compounds described below, and which it is desired to retain, may need to be in protected form before any reaction is initiated. In such instances, removal of the protecting group may be the final step in a particular reaction. Suitable amino or hydroxyl protecting groups include benzyl, benzyloxycarbonyl or t-butyloxycarbonyl groups. These may be removed from a protected derivative by catalytic hydrogenation using for example hydrogen in the presence of a metal catalyst, for example palladium on a support such as carbon in a solvent such as an alcohol e.g. methanol, or by treatment with trimethylsilyl iodide or trifluoroacetic acid in an aqueous solvent. Suitable carboxyl protecting groups include benzyl groups, which may be removed from a protected derivative by the methods just discussed, or alkyl groups, such as a t-butyl group which may be removed from a protected derivative by treatment with trifluoroacetic acid in an aqueous solvent. Other suitable protecting groups and methods for their use will be readily apparent. The formation of the protected amino, hydroxyl or carboxyl group may be achieved using standard alkylation or esterification procedures, for example as described below.

40 Thus according to a further aspect of the invention a compound of formula (I) may be prepared by coupling an acid of formula (II)



50 or an active derivative thereof, with an amine of formula (III)

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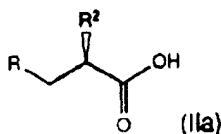
followed by removal of any protecting groups.

Active derivatives of acids for formula (II) include for example acid anhydrides, or acid halides, such as acid chlorides.

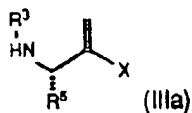
The coupling reaction may be performed using standard conditions for amination reactions of this type. Thus, for example the reaction may be achieved in a solvent, for example an inert organic solvent such as an ether, e.g. a cyclic ether such as tetrahydrofuran, an amide e.g. a substituted amide such as dimethylformamide, or a halogenated hydrocarbon such as dichloromethane at a low temperature, e.g. -30°C to ambient temperature, such as -20°C to 0°C, optionally in the presence of a base, e.g. an organic base such as an amine, e.g. triethylamine or a cyclic amine such as N-methylmorpholine. Where an acid of formula (II) is used, the reaction may additionally be performed in the presence of a condensing agent, for example a diimide such as N,N'-dicyclohexylcarbodiimide, advantageously in the presence of a triazole such as 1-hydroxybenzotriazole. Alternatively, the acid may be reacted with a chloroformate for example ethylchloroformate, prior to reaction with the amine of formula (III).

Free hydroxyl or carboxyl groups in the starting materials of formulae (II) [where R is -CONHOH or CO<sub>2</sub>H] and (III) may need to be protected during the coupling reaction. Suitable protecting groups and methods for their removal may be those mentioned above.

It will be appreciated that where a particular stereoisomer of formula (I) is required, this may be obtained by resolution of a mixture of isomers following the coupling reaction of an acid of formula (II) and an amine of formula (III). Conventional resolution techniques may be used, for example separation of isomers by Chromatography e.g. by use of high performance liquid chromatography. Where desired, however, appropriate homochiral starting materials may be used in the coupling reaction to yield a particular stereo isomer of formula (I). Thus, in particular process a compound of formula (Ia) may be prepared by reaction of a compound of formula (IIa)

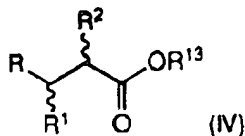


with an amine of formula (IIIa)



as described above

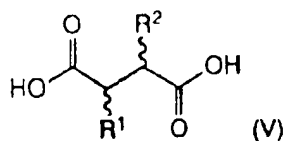
Intermediate acids of formula (II) wherein R is a carboxyl or esterified carboxyl group may be prepared by hydrolysing a corresponding ester of formula (IV)



where R<sup>13</sup> is an alkyl group, for example a methyl or t-butyl group, using for example trifluoroacetic acid, or,

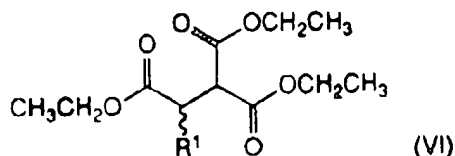
when R<sup>13</sup> is a methyl group using enzymatic hydrolysis, such as for example with  $\alpha$ -chymotrypsin, in an aqueous solvent. In this reaction, enzymatic hydrolysis (for example as more particularly described in the Examples herein) usefully provides a method of isomer selection.

The ester of formula (IV) may be prepared by esterification of the corresponding acid of formula (V)



using an appropriate acyl halide, for example an acyl chloride in a solvent such as an alcohol, e.g. methanol at a low temperature, e.g. around 0°C,

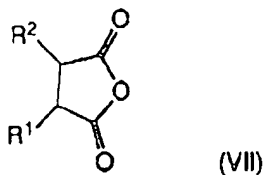
Acids of formula (V) may be prepared by alkylation of a compound of formula (VI)



with an appropriate halide, e.g. a compound  $R^2Hal$ , where Hal is a halogen atom such as a chlorine or bromine atom in the presence of a base, for example an alkoxide such as sodium ethoxide in a solvent such as an alcohol, e.g. ethanol at ambient temperature, followed by decarboxylation using for example concentrated hydrochloric acid at an elevated temperature, e.g. the reflux temperature.

Intermediates of formula (VI) are either known compounds or may be prepared by methods analogous to those used for the preparation of the known compounds.

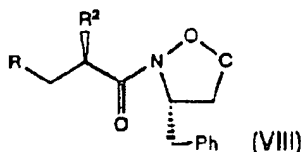
Intermediate acids of formula (IV) wherein R is a -CONHOH group or a protected derivative thereof may be prepared by reaction of an anhydride of formula (VII)



with a hydroxylamine such as O-benzylhydroxylamine in a solvent such as tetrahydrofuran at a low temperature, e.g. around -20°C, followed where desired by removal of the protecting group as described above.

The intermediate anhydrides of formula (VII) may be prepared for example by heating for example at the reflux temperature, a diacid of formula (V) where R is  $-CO_2H$  with an acyl chloride such an acetyl chloride.

The homochiral acids of formula (IIa) may be prepared according to another feature of the invention by oxidation of an oxazolidinone of formula (VIII)



(where Ph is a phenyl group)

using an oxidising agent such as peroxide, e.g. hydrogen peroxide in a solvent such as an ether e.g. a cyclic ether such as tetrahydrofuran, at a low temperature, e.g. around 0°C followed by treatment with a base, such

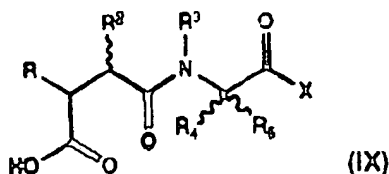
as lithium hydroxide, at an elevated temperature.

The compounds of formula (VIII) are novel, particularly useful, intermediates for the preparation of stereoisomers of formula (Ia) and form a further aspect of the invention.

The compounds of formula (VIII) may be prepared by reaction of an acyl halide  $RCH_2CH(R^2)COHal$  (where Hal is a halogen atom such as chloride, bromine or iodine atom) with a solution of (S)-4-(phenylmethyl)-2-oxazolidinone in the presence of a base such as n-butyl lithium in a solvent such as tetrahydrofuran at a low temperature, e.g. around  $-78^\circ C$ .

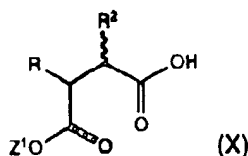
Acyl halides  $RCH_2CH(R^2)COHal$  may be prepared by treatment of the corresponding known acids  $RCH_2CH(R^2)CO_2H$  with conventional halogenating agents for example thionyl halides under standard reaction conditions.

In another process according to the invention, a compound of formula (I) where R is a carboxyl group may be prepared by decarboxylation of a corresponding compound of formula (IX).



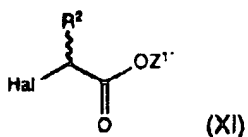
The reaction may be achieved using standard conditions, for example by heating a compound of formula (IX) in an inert solvent, such as an aromatic hydrocarbon, e.g. xylene, at the reflux temperature.

The intermediate acids of formula (IX) may be prepared by reaction of a protected acid of formula (X)



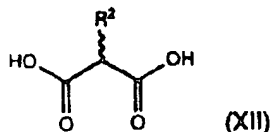
where R is a protected carboxyl group such as a benzyloxycarbonyl group and  $Z^1$  is a protecting group such as a benzyl group with an amine of formula (III) using reagents and conditions as described above for coupling compounds of formula (II) and (III), followed by removal of the protecting groups.

The intermediates of formula (X) may be prepared by treatment of an appropriate malonic ester  $RCH_2CO_2Z^1$  with a halide of formula (XI)



(where Hal is a halogen atom, e.g. a chlorine or bromine atom) in the presence of a base such as potassium t-butoxide in a solvent such as dimethylformamide at ambient temperature.

Halides of formula (XI) may be prepared by halogenation and subsequent decarboxylation of a di-acid of formula (XII).





using for example a halogenating agent such as bromine in a solvent such as diethyl ether at ambient temperature, followed by heating of the resulting halogenated intermediate in a solvent such as an aromatic hydrocarbon e.g. xylene, at the reflux temperature.

Intermediates of formula (XII) may be prepared by hydrolysis of the corresponding di-alkylester (e.g. the dimethyl or diethyl ester using a base such as sodium or potassium hydroxide in a solvent such as an alcohol e.g. methanol at the reflux temperature. The di-alkyl ester starting materials are either known compounds or may be prepared by methods analogous to those used for the preparation of the known compounds, for example as described in the Examples herein.

Compounds of formula (I) may also be prepared by interconversion of other compounds of formula (I). Thus, for example, a compound of formula (I) wherein R is a -CONHOH group may be prepared by reaction of a corresponding acid of formula (I) wherein R is a -CO<sub>2</sub>H group or an active derivate thereof (for example an acid chloride or an acid anhydride) with hydroxylamine or an O-protected derivative or a salt thereof. The reaction may be performed using the reagents and conditions described above in the preparation of compounds of formula (I) from the starting materials of formulae (II) and (III).

In another interconversion process, compounds of formula (I) wherein R is -CO<sub>2</sub>H and/or X contains a -CO<sub>2</sub>H group may be prepared by hydrolysis of the corresponding esterified compounds (for example where R is a -CO<sub>2</sub>R<sup>8</sup> group and/or X contains a similar group) using conventional procedures, for example by treatment with a base, e.g. an alkali metal hydroxide such as lithium hydroxide in a solvent such as an aqueous alcohol, e.g. aqueous methanol, or by treatment with an acid such as a mineral acid, e.g. hydrochloric acid in the presence of a solvent, e.g. dioxan.

Similarly esters of formula (I), for example where R is a CO<sub>2</sub>R<sup>8</sup> group and/or X contains a -CO<sub>2</sub>R<sup>8</sup> group may be prepared by reaction of the corresponding acids, where R is a -CO<sub>2</sub>H group and/or X contains a -CO<sub>2</sub>H group or an active derivative thereof, with an alcohol R<sup>8</sup>OH using standard conditions.

The compounds according to the invention are potent and selective inhibitors of gelatinase. The activity and selectivity of the compounds may be determined by the use of appropriate enzyme inhibition test for example as described in Example A hereinafter. In our tests using this approach, compounds according to the invention have been shown to inhibit gelatinase with K<sub>i</sub> values in the picomolar-nanomolar range and to have around a 40 fold or greater selectivity for gelatinase over stromelysin, and around a 20-fold or greater selectivity for gelatinase over collagenase.

The ability of compounds of the invention to prevent tumour cell invasion may be demonstrated in a standard mouse model.

Thus, briefly, nude mice may be inoculated with a tumour cell line showing gelatinase - dependent invasion and the ability of compounds according to the invention to reduce subsequent lung tumour colonisation may be evaluated in accordance with standard procedures. In our tests, compounds according to the invention, when administered intravenously at 1 mg/kg to mice in the above model have reduced lung tumour colonisation to negligible levels.

The compounds according to the invention can be expected to be of use to prevent tumour cell metastasis and invasion. The compounds may therefore be of use in the treatment of cancer, particularly in conjunction with radiotherapy, chemotherapy or surgery, or in patients presenting with primary tumours, to control the development of tumour metastasises. Thus, according to a further aspect of the invention we provide a compound of formula (I) for use in the treatment of cancer to control the development of tumour metastasises. Particularly cancers may include breast, melanoma, lung, head, neck or bladder cancers.

For use according to this aspect of the invention, the compounds of formula (I) may be formulated in a conventional manner, optionally with one or more physiologically acceptable carriers, diluents or excipients.

Thus according to a further aspect of the invention we provide a pharmaceutical composition comprising a compound of formula (I) and a pharmaceutically acceptable diluent, carrier or excipient.

In a still further aspect the invention provides a process for the production of a pharmaceutical composition comprising bringing a compound of formula (I) into association with a pharmaceutically acceptable diluent, carrier or excipient.

Compounds for use according to the present invention may be formulated for oral, buccal, parental or rectal administration or in a form suitable for nasal administration or administration by inhalation or insufflation.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g. pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (e.g. lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (e.g. magnesium stearate, talc or silica); disintegrants (e.g. potato starch or sodium glycolate); or wetting agents (e.g. sodium lauryl sulphate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitu-

tion with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents, emulsifying agents, non-aqueous vehicles; and preservatives. The preparations may also contain buffer salts, flavouring, colouring and sweetening agents as appropriate.

5 Preparations for oral administration may be suitably formulated to give controlled release of the active compound.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

10 The compounds of formula (I) may be formulated for parental administration by injection e.g. by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form. The compositions for injection may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile pyrogen-free water, before use.

15 The compounds of formula (I) may also be formulated in rectal compositions such as suppositories or retention enemas, e.g. containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described above the compounds of formula (I) may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation or by intramuscular injection.

20 For nasal administration or administration by inhalation the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation for pressurised packs or a nebuliser, with the use of suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas.

25 The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack or dispenser device may be accompanied by instructions for administration.

The doses of compounds of formula (I) used to control the development of tumour metastasises will vary depending on the condition of the patient to be treated but in general may be in the range around 0.5mg to 50mg/kg body weight, particularly from about 1mg to 40mg/kg body weight. Dosage units may be varied according to the route of administration of the compound in accordance with conventional practice.

#### Description of Specific Embodiments

The invention is further illustrated in the following non-limiting Examples.

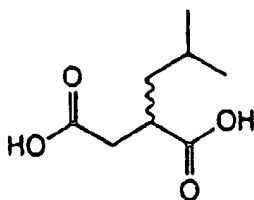
35 In the Examples, the following abbreviations are used:

RT - room temperature  
 DCCI - N,N'-dicyclohexylcarbodiimide  
 DMF - dimethylformamide  
 THF - tetrahydrofuran  
 40 TFA - trifluoroacetic acid  
 RPHPLC reverse phase high performance liquid chromatography  
 HOBT - N-hydroxybenzotriazole

#### Reference Example

45 [4-N-(Hydroxyamino)-2R-isobutylsuccinyl]-L-valine-L-alanine amide

R.S - Isobutylsuccinic acid K

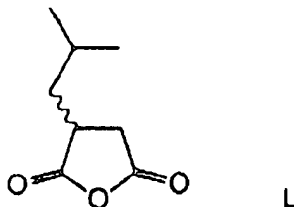


K

Sodium ethoxide was prepared by adding sodium metal (2.5g, 108mmol) to anhydrous ethanol (150ml) under nitrogen. Triethyl 1,1,2-ethanetricarboxylate (26.6g, 25ml, 108mmol) was added and the mixture stirred at room temperature (RT) for 20 minutes. Isobutyl bromide (19.12g, 15ml, 108mmol) was added dropwise over 1 hour and the solution raised to reflux overnight. The precipitated sodium bromide was filtered off and the filtrate concentrated *in vacuo*. The residue was treated with cold H<sub>2</sub>O (200ml) and extracted with diethyl ether (3 × 100ml). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to give a clear oil (32.2g) which was refluxed with concentrated hydrochloric acid for 96 hours. On cooling, a white crystalline solid precipitated which was filtered, washed with ice cold water and dried *in vacuo* to give the title compound K (11.0g)

<sup>1</sup>HNMR (CDCl<sub>3</sub>) δ 0.85 (3H, d, J = 6Hz), 0.90 (3H, d, J=6Hz), 1.3-1.45 (1H, m), 1.55-1.75 (2H, m), 2.50 (1H, dd, J=6 and 18 Hz), 2.70 (1H, dd, J=9 and 18 Hz), 2.85-2.95 (1H, m).

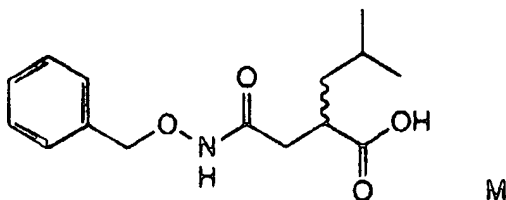
### 3-(R,S)-Isobutylsuccinic anhydride L



The diacid K (10.21g, 59mmol) was treated with acetyl chloride (27ml, 376mmol) under reflux for 2.1/2 hours. Volatiles were removed under reduced pressure to give the anhydride L (9.37g, 100%) as a brownish oil.

<sup>1</sup>HNMR (CDCl<sub>3</sub>) 0.95 (3H, d, J = 6Hz), 1.05 (3H, d, J=6Hz), 1.48-1.90 (3H, m), 2.65 (1H, dd, J=7 and 18 Hz), 3.10 (1H, dd, J=9 and 18 Hz), 3.15-3.25 (1H, m).

### [4-(N-Benzoyloxyamino)-2 R,S-Isobutyl] succinic acid M



O-Benzyl hydroxylamine (7.8g, 63.4 mmol) in dry THF (50ml) was added dropwise (over 1 hour) to a solution of the anhydride L (9.37g, 60.0mmol) in dry THF (100ml) at -20°C. After stirring a further 1 hour, volatiles were removed *in vacuo* and the residue taken up in ethyl acetate. After washing with 1.0MHCL ×3, the organic phase was dried (MgSO<sub>4</sub>) and evaporated to give a white solid. The crude solid was dissolved in hot diethyl ether and filtered. Colourless crystals of the acid M deposited on standing (6.7g, 41%).

<sup>1</sup>HNMR (CDCl<sub>3</sub>) δ 0.8-1.0 (6H, m), 1.2-1.4 (3H, m), 2.1-2.4 (2H, m), 2.8-3.0 (1H, m), 4.85 (2H, s), 7.3 (5H,bs), 8.6 (1H, bs).

### [4-N-(Hydroxyamino)-2R-isobutylsuccinyl]-L-valine-L-alanine amide

The acid M (502mg, 1.8mmol) was dissolved in dry THF (20ml) and cooled to -20°C. Ethylchloroformate (245mg, 233μl, 1.8mmol) and N-methyl morpholine was added and the suspension left for 1 hour at -20°C. A DMF solution (10ml) of L-valine-L-alanine amide (500mg) was added dropwise. Once the addition was completed the cooling bath was removed and the reaction allowed to warm up to room temperature overnight. The organic solution was poured into 10% HCl and extracted with ethyl acetate (×3). The organic layer was dried (MgSO<sub>4</sub>) and concentrated *in vacuo* to give a solid.

The solid was dissolved in degassed MeOH (20ml) and hydrogenolysed using 5% Pd-C and hydrogen gas. After 1 hour at RT the catalyst was filtered off and the product purified on RPHPLC using 0.1%TFA/H<sub>2</sub>O→

0.1%TFA/CH<sub>3</sub>CN (43:57) isocratically to yield the title compound.

<sup>1</sup>HNMR (CD<sub>3</sub>OD) δ 0.95(12H, m), 1.35(3H, d, J=6Hz), 1.95(3H, m), 2.20 (1H, m), 2.35(1H, m) 2.85(2H,m), 4.35(2H, m)

The following compounds of Examples 1-14 were prepared in a similar manner to the Reference Compound using the appropriate analogous starting materials

#### Example 1

[4-(N-Hydroxyamino)-2R-phenylethylsuccinyl]-L-leucine-N-(2-phenylethyl)amide

<sup>1</sup>HNMR (CD<sub>3</sub>OD) 7.15-7.30(10H, mult, Ar); 4.40(1H, mult, NCHCO); 3.35-3.55 (2H, mult, CH<sub>2</sub>N); 2.20-2.85(7H, mult, CHCO + CH<sub>2</sub>Ar); 1.50-1.95(5H, mult, CHC) 0.95(6H, dd, CH<sub>3</sub>)

#### Example 2

[4-(N-Hydroxyamino)-2R-phenylpropylsuccinyl]-L-leucine-N-(2-phenylethyl)amide

<sup>1</sup>HNMR (CD<sub>3</sub>OD) 7.1-7.3(10H, mult, Ar); 4.30(1H, dd, NCHCO); 3.35 (2H, mult, CH<sub>2</sub>N); 2.15-2.80(7H, mult, CHCO+CH<sub>2</sub>Ar); 1.5-1.75(7H, mult, CHC); 0.95(6H, dd, CH<sub>3</sub>)

#### Example 3

[4-(N-Hydroxyamino)-2(R)-isobutylsuccinyl]-L-tryptophan amide

<sup>1</sup>HNMR (CD<sub>3</sub>OD) δ 7.65(1H, d); 7.35(1H, d); 6.95-7.15(3H, m); 4.65(1H, dd), 3.05-3.2(1H, m), 2.7-2.85(2H, m), 2.0-2.2(2H, m), 1.3-1.5(2H, m) 1.05-1.15(1H, m) 0.7(3H, d), 0.65(3H, d)

#### Example 4

[4-(N-Hydroxyamino) 2(R)-isobutylsuccinyl]-L-valine amide

<sup>1</sup>HNMR (CD<sub>3</sub>OD) δ 4.15(1H, d); 2.8-2.9(1H, m); 2.2-2.35(1H, m); 1.95-2.15(2H, m), 1.45-1.55(2H, m), 1.1-1.2(1H, m), 0.8-1.05(12H, m)

#### EXAMPLE A

The activity and selectivity of the compounds of the invention may be determined as described below.

All enzyme assays to determine K<sub>i</sub> values were performed using the peptide substrate Dnp-Pro-Leu-Gly-Leu-Trp-Ala-D-Arg-NH<sub>2</sub> [M. Sharon Stock and Robert D. Gray. JBC 264, 4277-81, 1989). The enzymes cleave at the Gly-Leu bond which can be followed fluorimetrically by measuring the increase in Trp fluorescence emission associated with the removal of the quenching dinitrophenol (Dnp) group.

Essentially, enzyme (e.g. gelatinase, stromelysin, collagenase) at 0.08-2nM; a range of inhibitor concentrations (0.1-50 × K<sub>i</sub>) and substrate (approx. 20μM) are incubated overnight in 0.1M Tris/HCl buffer, pH 7.5, containing 0.1M NaCl, 10mM CaCl<sub>2</sub> and 0.05% Brij 35 at either room temperature or 37°C depending on the enzyme. The reaction is stopped by adjusting the pH to 4 using 0.1M sodium acetate buffer and the fluorescence read at an excitation wavelength of 280nm and emission wavelength of 346nm.

K<sub>i</sub> values can be established using the equation for tight-binding inhibition:-

$$V_i = \frac{V_o}{2[E]} \left( \sqrt{(K_{i(app)} + [I]^2 + 2(K_{i(app)} - [I])[E] + [E]^2 - (K_{i(app)} + [I] \cdot [E])} \right)$$

where V<sub>o</sub> is the initial rate of reaction in the absence of inhibitor, V<sub>i</sub> is the initial rate in the presence of inhibitor, [E] is the total enzyme concentration and [I] the total inhibitor concentration in the reaction mixture.

For stromelysin and collagenase, K<sub>i</sub>(app) was assumed to approximate to the true K<sub>i</sub> as [S] << K<sub>m</sub> for the

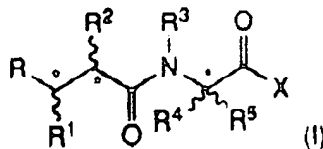
substrate hydrolysis. For gelatinase the  $k_j$  was determined by performing the analyses at several substrate concentrations. A plot of  $K_j(\text{app})$  vs.  $[S]$  then gave the true  $K_j$  as the value of the y-axis intercept

The following results were obtained with compounds according to the invention.

	<u><math>K_i</math> (nM)</u>		
	Collagenase	Stromelysin-1	Gelatinase -72KD
<u>Reference Compound</u>	303	129	33
<u>Compound of Example 2</u>	79	16	0.17

## Claims

1. A compound of formula (I):



wherein R represents a -CONHOH, carboxyl (-CO<sub>2</sub>H) or esterified carboxyl group;

R<sup>1</sup> represents an optionally substituted alkyl, alkenyl, aryl, aralkyl, heteroaralkyl or heteroarylthioalkyl group;

R<sup>2</sup> represents an optionally substituted phenylethyl phenylpropyl or phenylbutyl group;

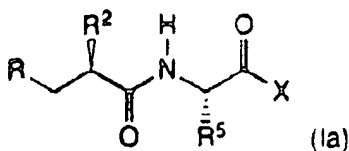
R<sup>3</sup> represents a hydrogen atom or an alkyl group;

R<sup>4</sup> represents a hydrogen atom or an alkyl group;

R<sup>5</sup> represents an optionally substituted alkyl or alkenyl group optionally interrupted by one or more -O- or -S- atoms or -N(R<sup>7</sup>)- groups [where R<sup>7</sup> is a hydrogen atom or a C<sub>1-6</sub>alkyl group];

X represents an amino (-NH<sub>2</sub>), or substituted amino, hydroxyl or substituted hydroxyl group ;  
and the salts, solvates and hydrates thereof.

2. A compound according to Claim 1 wherein R represents a -CONHOH or carboxyl (-CO<sub>2</sub>H) group.
3. A compound according to claim 1 or 2 wherein R<sup>1</sup>, R<sup>3</sup> and R<sup>4</sup> is each a hydrogen atom
4. A compound according to any of Claims 1-3 wherein R<sup>2</sup> is an optionally substituted phenylpropyl group.
5. A compound according to any of the preceding claims wherein X is an amino or substituted amino group.
6. A compound of formula (Ia)



wherein R represents a -CONHOH, carboxyl (-CO<sub>2</sub>H) or esterified carboxyl group;

R<sup>2</sup> represents an optionally substituted phenylpropyl group

R<sup>5</sup> represents an optionally substituted alkyl or alkenyl group optionally interrupted by one or more -O- or -S- atoms or -N(R<sup>7</sup>)- groups [where R<sup>7</sup> is a hydrogen atom or C<sub>1-6</sub>alkyl group]

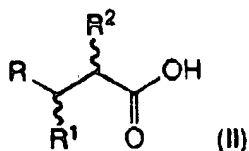
5 X represents an amino (-NH<sub>2</sub>), or substituted amino, hydroxyl or substituted hydroxyl group; and the salts, solvates and hydrates thereof

7. A pharmaceutical composition comprising a compound according to any one Claims 1 to 6 and a pharmaceutical diluent, carrier or excipient.

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8. A process for preparing a compound of formula (I) as defined in Claim 1, the process comprising:  
(a) coupling an acid of formula (II)

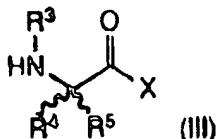
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or an active and/or protected derivative thereof, with an amine of formula (III)

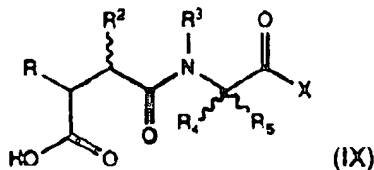
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or a protected derivative thereof followed by removal of any protecting groups; or  
(b) decarboxylating a compound of formula (IX)

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to produce a compound of formula (I) wherein R is a -CO<sub>2</sub>H group; and/or  
(c) interconverting a compound of formula (I)

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European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number

EP 91 31 1254

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A,D	EP-A-0 214 639 (G.D. SEARLE & CO.) * page 7 - page 14; claims; examples 13,14 *	1-8	C07C259/06 A61K31/16
A,D	WO-A-9 005 716 (BRITISH BIO-TECHNOLOGY LIMITED) * claims *	1-8	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			C07C
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 09 MARCH 1992	Searcher SANCHEZ Y GARCIA J.
<p><b>CATEGORY OF CITED DOCUMENTS</b></p> <p>X : particularly relevant if taken alone  Y : particularly relevant if combined with another document of the same category  A : technological background  O : non-written disclosure  P : intermediate document</p> <p>T : theory or principle underlying the invention  E : earlier patent document, but published on, or after the filing date  D : document cited in the application  L : document cited for other reasons  Δ : member of the same patent family, corresponding document</p>			

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